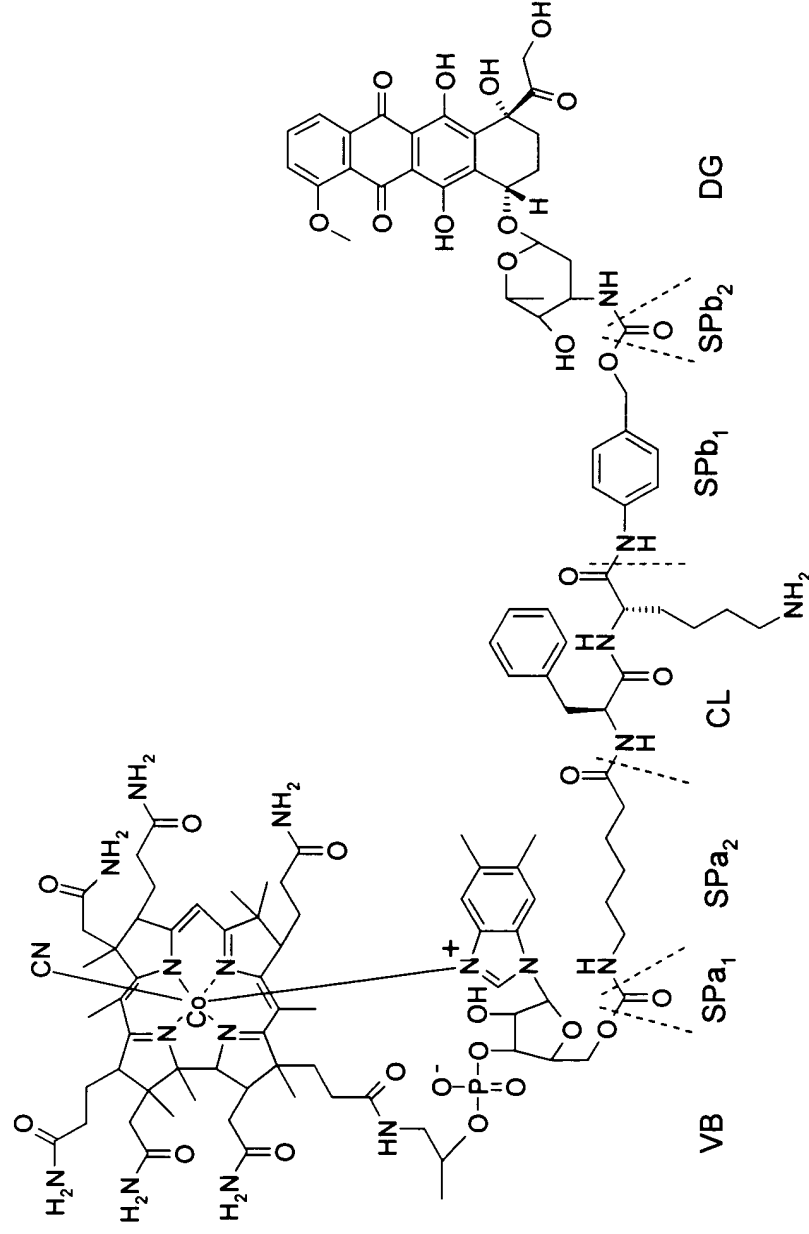FIG. 1a
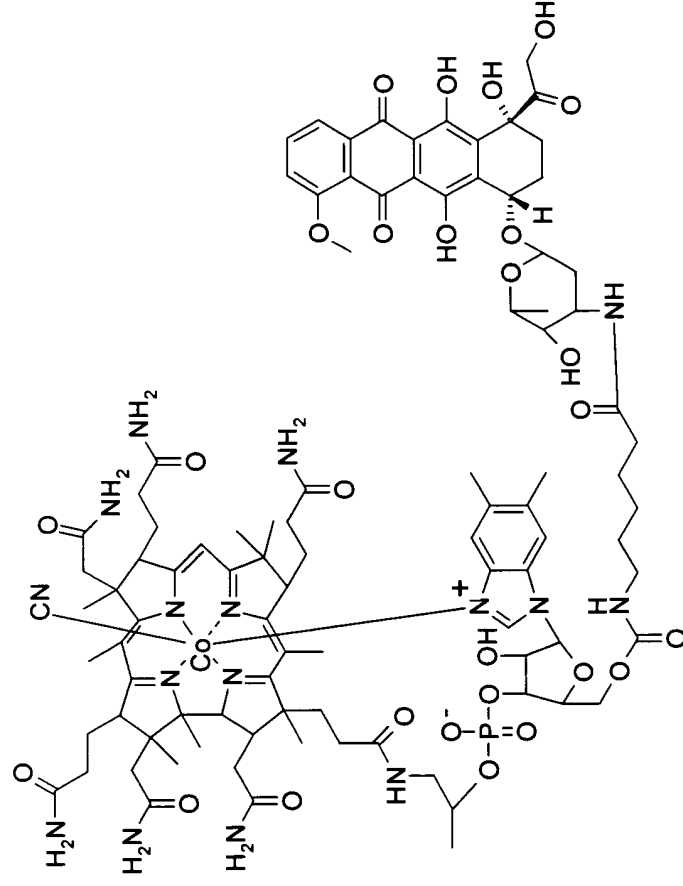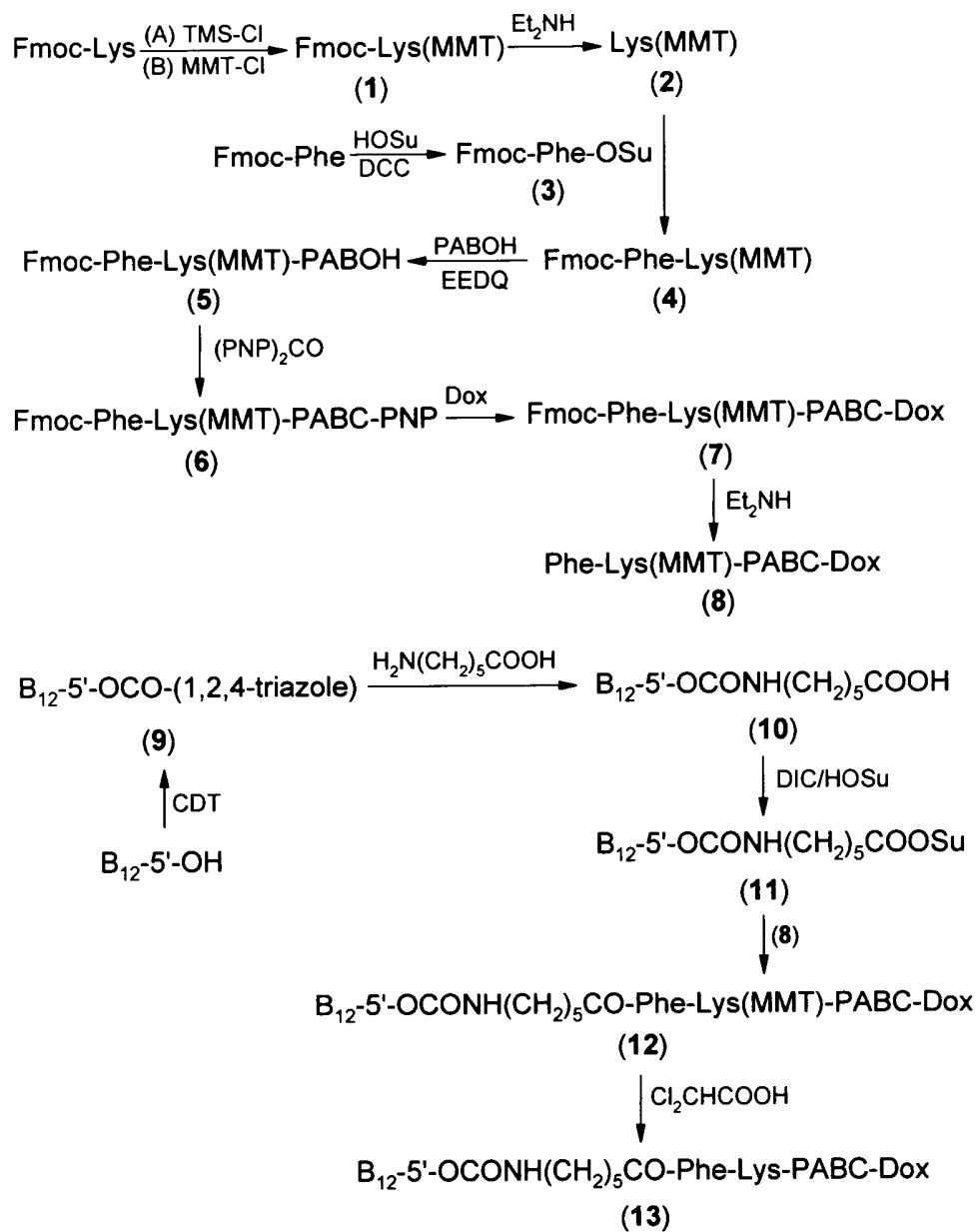

DG

SPb₂

SPb₁

CL

NH₂

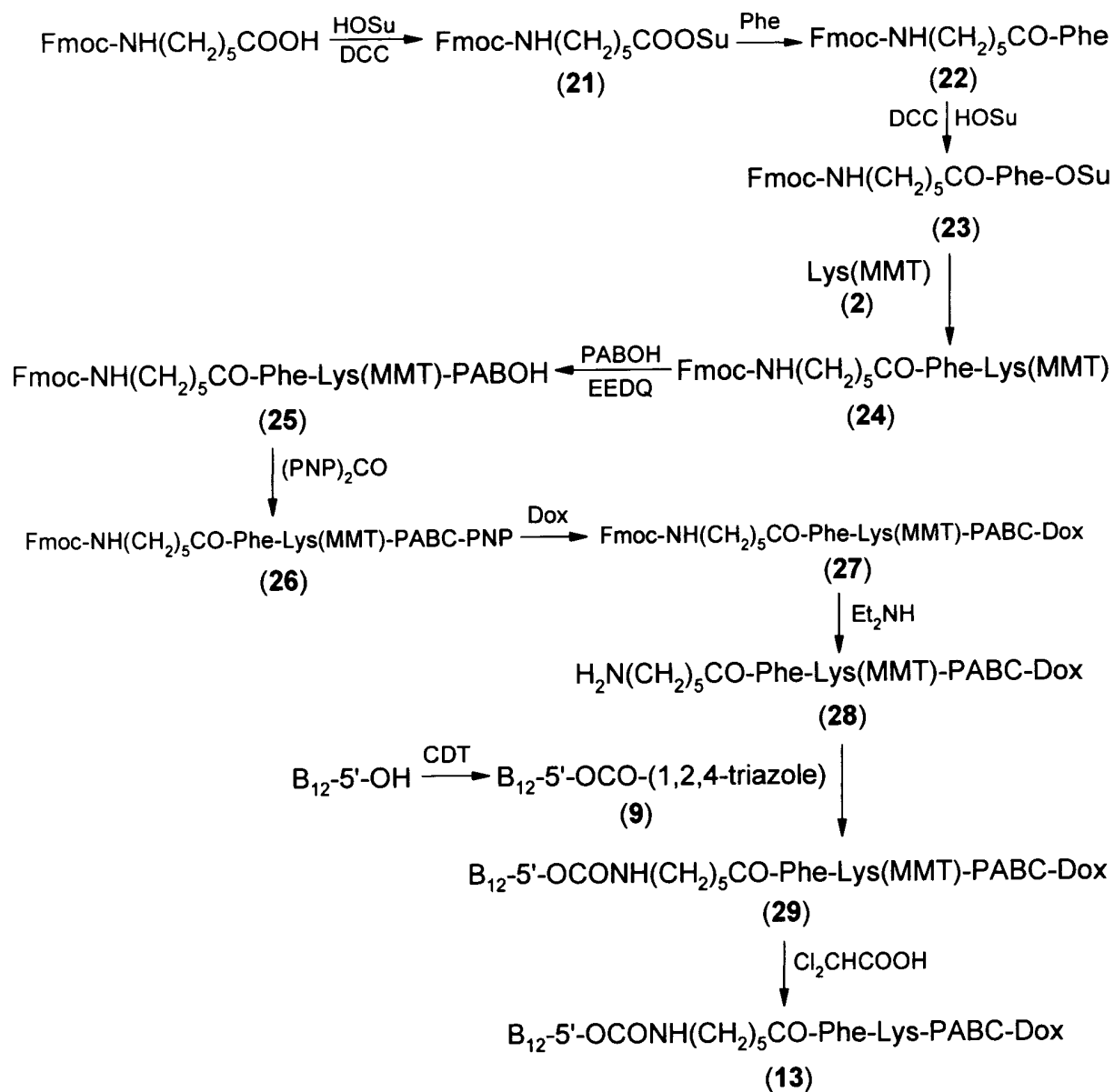SPa₂   SPa₁

VB

13

Cathepsin B-cleavable B12-Dox conjugate

FIG. 1b

Stable B12-Dox conjugate

$Fmoc\text{-}Lys \xrightarrow[\text{(B) MMT-Cl}]{\text{(A) TMS-Cl}} Fmoc\text{-}Lys(MMT) \xrightarrow{Et_2NH} Lys(MMT)$

(1)   **(2)**

$Fmoc\text{-}Phe \xrightarrow[\text{DCC}]{\text{HOSu}} Fmoc\text{-}Phe\text{-}OSu$

(3)

$Fmoc\text{-}Phe\text{-}Lys(MMT)\text{-}PABOH \xleftarrow[\text{EEDQ}]{\text{PABOH}} Fmoc\text{-}Phe\text{-}Lys(MMT)$

(5)   **(4)**

$\downarrow (PNP)_2CO$

$Fmoc\text{-}Phe\text{-}Lys(MMT)\text{-}PABC\text{-}PNP \xrightarrow{Dox} Fmoc\text{-}Phe\text{-}Lys(MMT)\text{-}PABC\text{-}Dox$

(6)   **(7)**

$\downarrow Et_2NH$

$Phe\text{-}Lys(MMT)\text{-}PABC\text{-}Dox$

(8)

$B_{12}\text{-}5'\text{-}OCO\text{-}(1,2,4\text{-}triazole) \xrightarrow{H_2N(CH_2)_5COOH} B_{12}\text{-}5'\text{-}OCONH(CH_2)_5COOH$

(9)   **(10)**

$\uparrow CDT$ $\downarrow DIC/HOSu$

$B_{12}\text{-}5'\text{-}OH$ $B_{12}\text{-}5'\text{-}OCONH(CH_2)_5COOSu$

(11)

$\downarrow (8)$

$B_{12}\text{-}5'\text{-}OCONH(CH_2)_5CO\text{-}Phe\text{-}Lys(MMT)\text{-}PABC\text{-}Dox$

(12)

$\downarrow Cl_2CHCOOH$

$B_{12}\text{-}5'\text{-}OCONH(CH_2)_5CO\text{-}Phe\text{-}Lys\text{-}PABC\text{-}Dox$

(13)

$$Fmoc\text{-}NH(CH_2)_5COOH \xrightarrow[DCC]{HOSu} Fmoc\text{-}NH(CH_2)_5COOSu \xrightarrow{Phe} Fmoc\text{-}NH(CH_2)_5CO\text{-}Phe$$

(21)

(22)

DCC | HOSu

$$Fmoc\text{-}NH(CH_2)_5CO\text{-}Phe\text{-}OSu$$

(23)

Lys(MMT)
(2)

$$Fmoc\text{-}NH(CH_2)_5CO\text{-}Phe\text{-}Lys(MMT)\text{-}PABOH \xleftarrow[EEDQ]{PABOH} Fmoc\text{-}NH(CH_2)_5CO\text{-}Phe\text{-}Lys(MMT)$$

(25)

(24)

| (PNP)$_2$CO

$$Fmoc\text{-}NH(CH_2)_5CO\text{-}Phe\text{-}Lys(MMT)\text{-}PABC\text{-}PNP \xrightarrow{Dox} Fmoc\text{-}NH(CH_2)_5CO\text{-}Phe\text{-}Lys(MMT)\text{-}PABC\text{-}Dox$$

(26)

(27)

| Et$_2$NH

$$H_2N(CH_2)_5CO\text{-}Phe\text{-}Lys(MMT)\text{-}PABC\text{-}Dox$$

(28)

$$B_{12}\text{-}5'\text{-}OH \xrightarrow{CDT} B_{12}\text{-}5'\text{-}OCO\text{-}(1,2,4\text{-}triazole)$$

(9)

$$B_{12}\text{-}5'\text{-}OCONH(CH_2)_5CO\text{-}Phe\text{-}Lys(MMT)\text{-}PABC\text{-}Dox$$

(29)

| Cl$_2$CHCOOH

$$B_{12}\text{-}5'\text{-}OCONH(CH_2)_5CO\text{-}Phe\text{-}Lys\text{-}PABC\text{-}Dox$$

(13)

Taxol

MMT-Cl
pyridine

Taxol-2'-MMT (41)

Cl₃COCOCl
pyridine/DIEA

Taxol-2'-MMT-7-OCOCl (42)

Fmoc-Phe-Lys(MMT)-PABOH (5)

Fmoc-Phe-Lys(MMT)-PABC-7-Taxol-2'-MMT
(43)

DBU

Phe-Lys(MMT)-PABC-7-Taxol-2'-MMT
(44)

B₁₂-5'-OCONH(CH₂)₅COOSu (11)

B₁₂-5'-OCONH(CH₂)₅CO-Phe-Lys(MMT)-PABC-7-Taxol-2'-MMT
(45)

Cl₂CHCOOH

B₁₂-5'-OCONH(CH₂)₅CO-Phe-Lys-PABC-7-Taxol
(46)

Fmoc-Phe-Lys(MMT)-PABC-PNP

(**6**)

$\downarrow$ Taxol

Fmoc-Phe-Lys(MMT)-PABC-2'-Taxol

(**14**)

$\downarrow$ DBU

Phe-Lys(MMT)-PABC-2'-Taxol

(**15**)

$\downarrow$ (**11**)

B_{12}-5'-OCONH$(CH_2)_5$CO-Phe-Lys(MMT)-PABC-2'-Taxol

(**16**)

$\downarrow$ Cl_2CHCOOH

B_{12}-5'-OCONH$(CH_2)_5$CO-Phe-Lys-PABC-2'-Taxol

(**17**)

FIG. 5

B_{12}-5'-OH + O=N—⟨ ⟩—COOCH$_2$CH$_3$ ⟶ B_{12}-5'-OCONH—⟨ ⟩—COOCH$_2$CH$_3$

(51)

$\downarrow$ K$_2$CO$_3$

B_{12}-5'-OCONH—⟨ ⟩—COOH

(52)

$\downarrow$ SuOH/DCC

B_{12}-5'-OCONH—⟨ ⟩—COOSu

(53)

$\downarrow$ Phe-Lys(MMT)-PABC-Dox (8)

B_{12}-5'-OCONH—⟨ ⟩—CO-Phe-Lys(MMT)-PABC-Dox

(54)

$\downarrow$ Cl$_2$CHCOOH

B_{12}-5'-OCONH—⟨ ⟩—CO-Phe-Lys(MMT)-PABC-Dox

(55)

Viability of MCF-7 cells treated for 96 hours
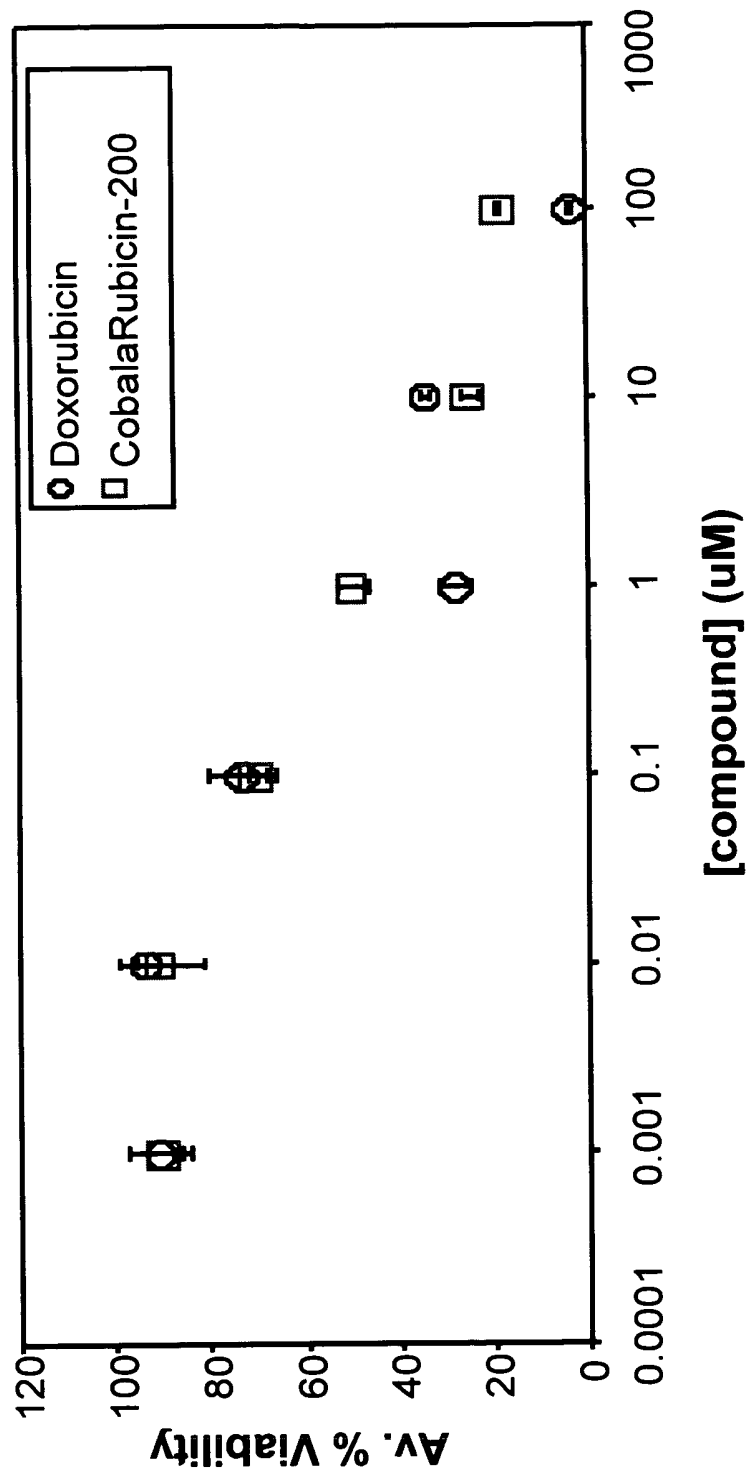
Viability of SK-BR-3 cells treated for 96 hours

● Doxorubicin

■ CobalaRubicin-200

Av. % Viability

[compound] (uM)
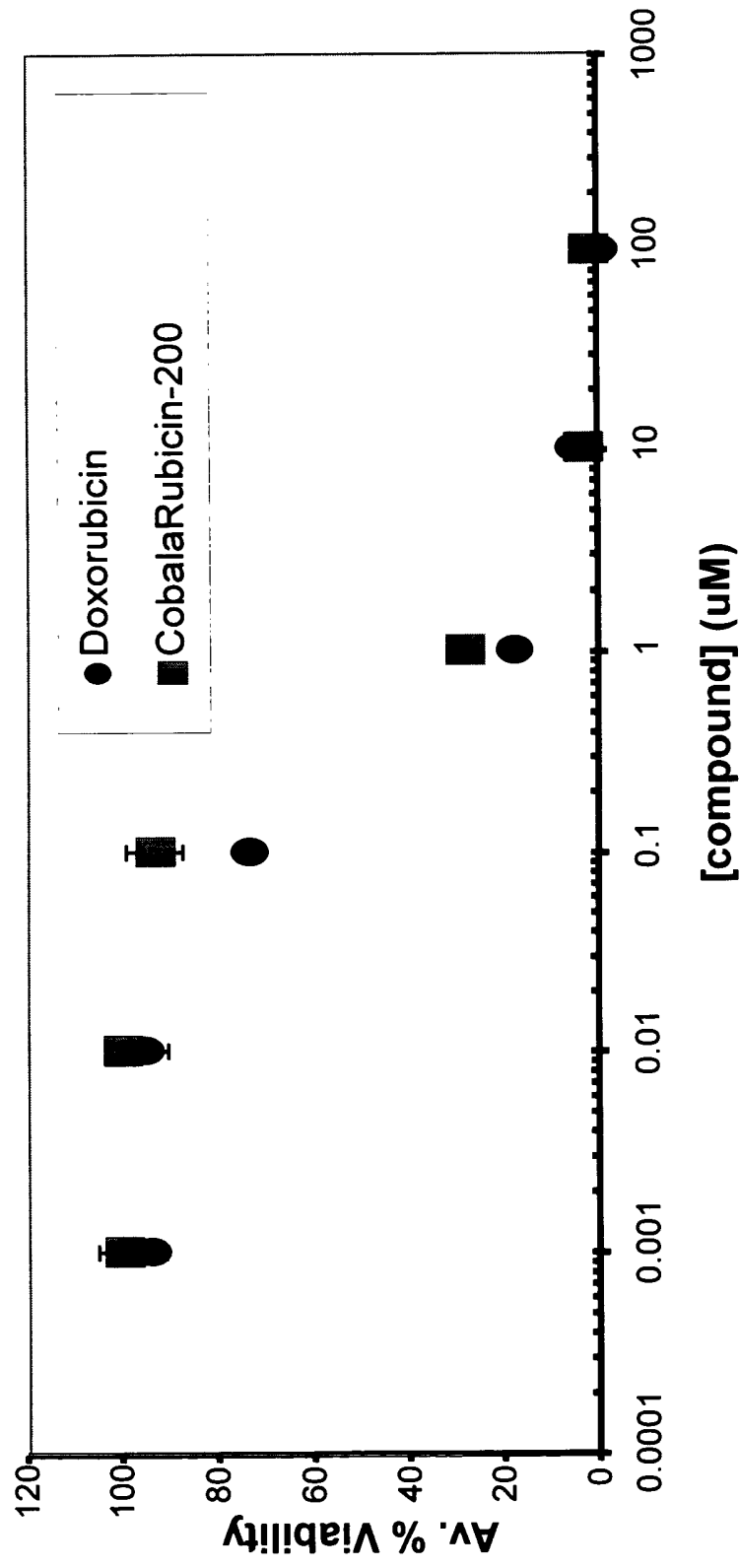
Viability of HL-60 cells (promyelocytic leukemia) treated for 96 hours
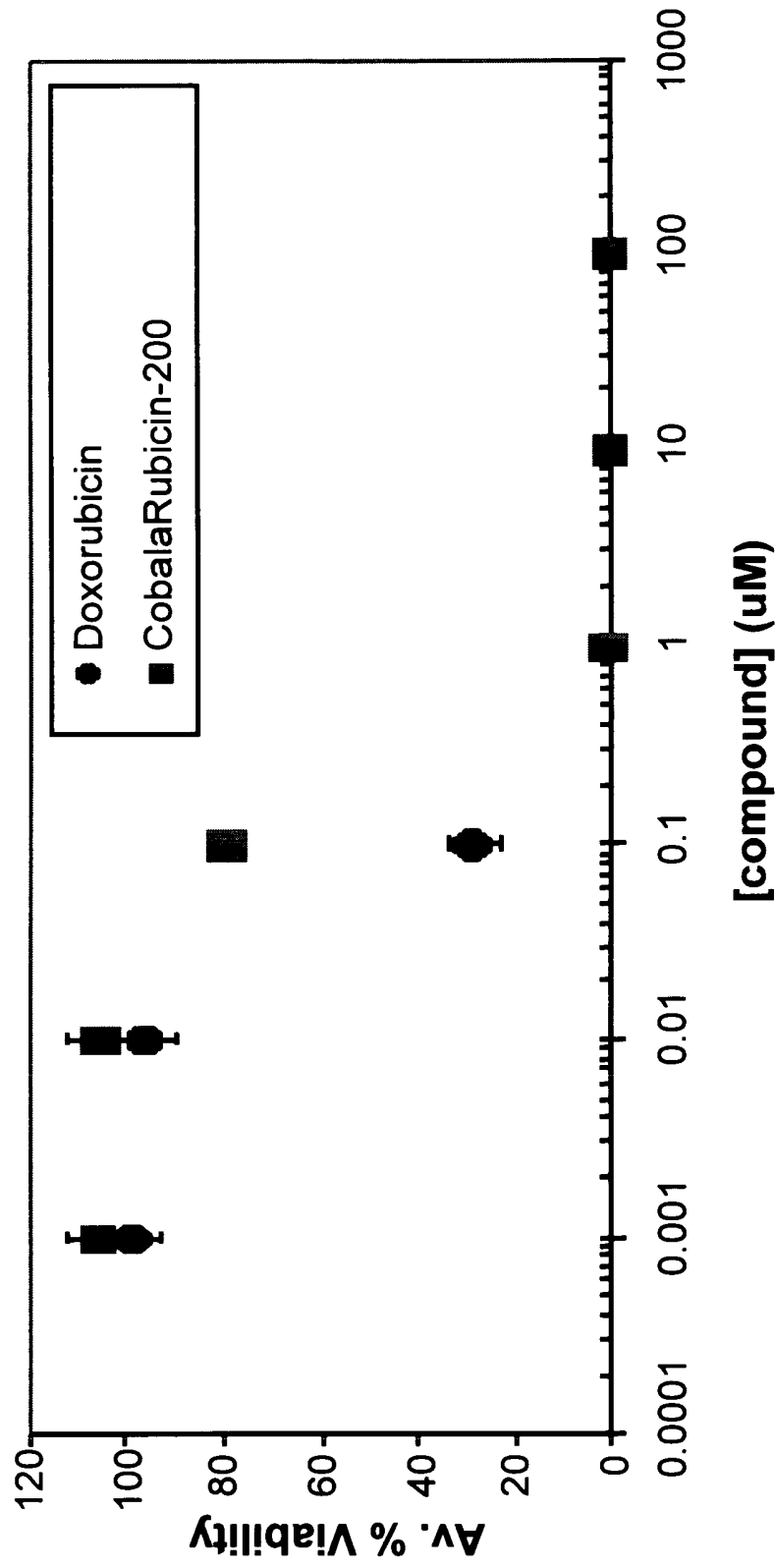
FIG. 6d

Viability of SK-N-MC cells treated for 48 hours
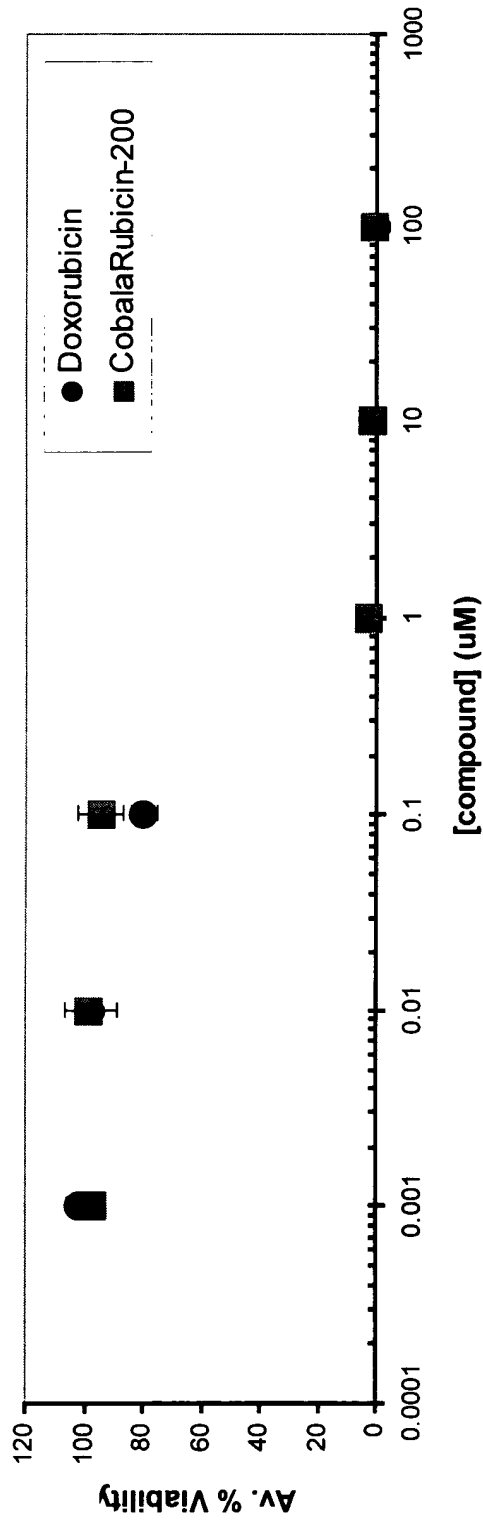
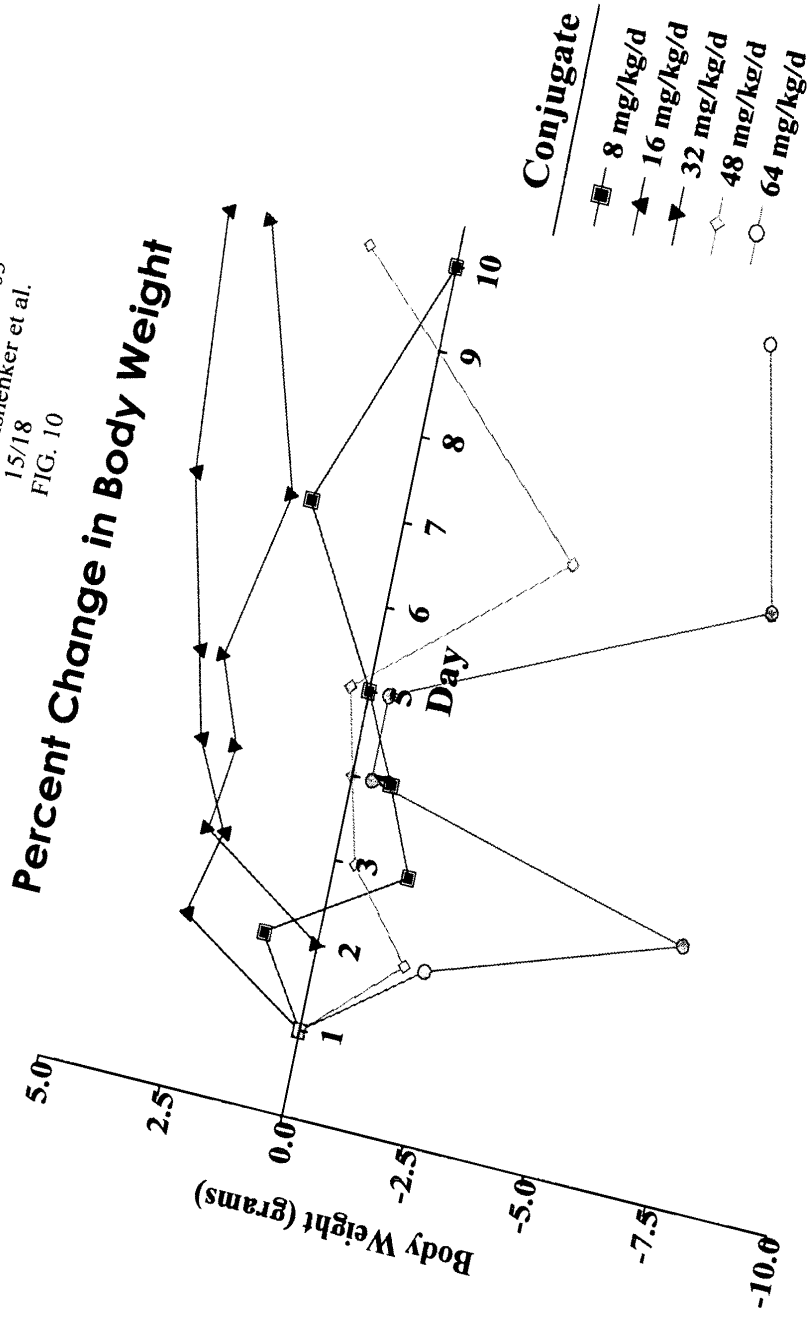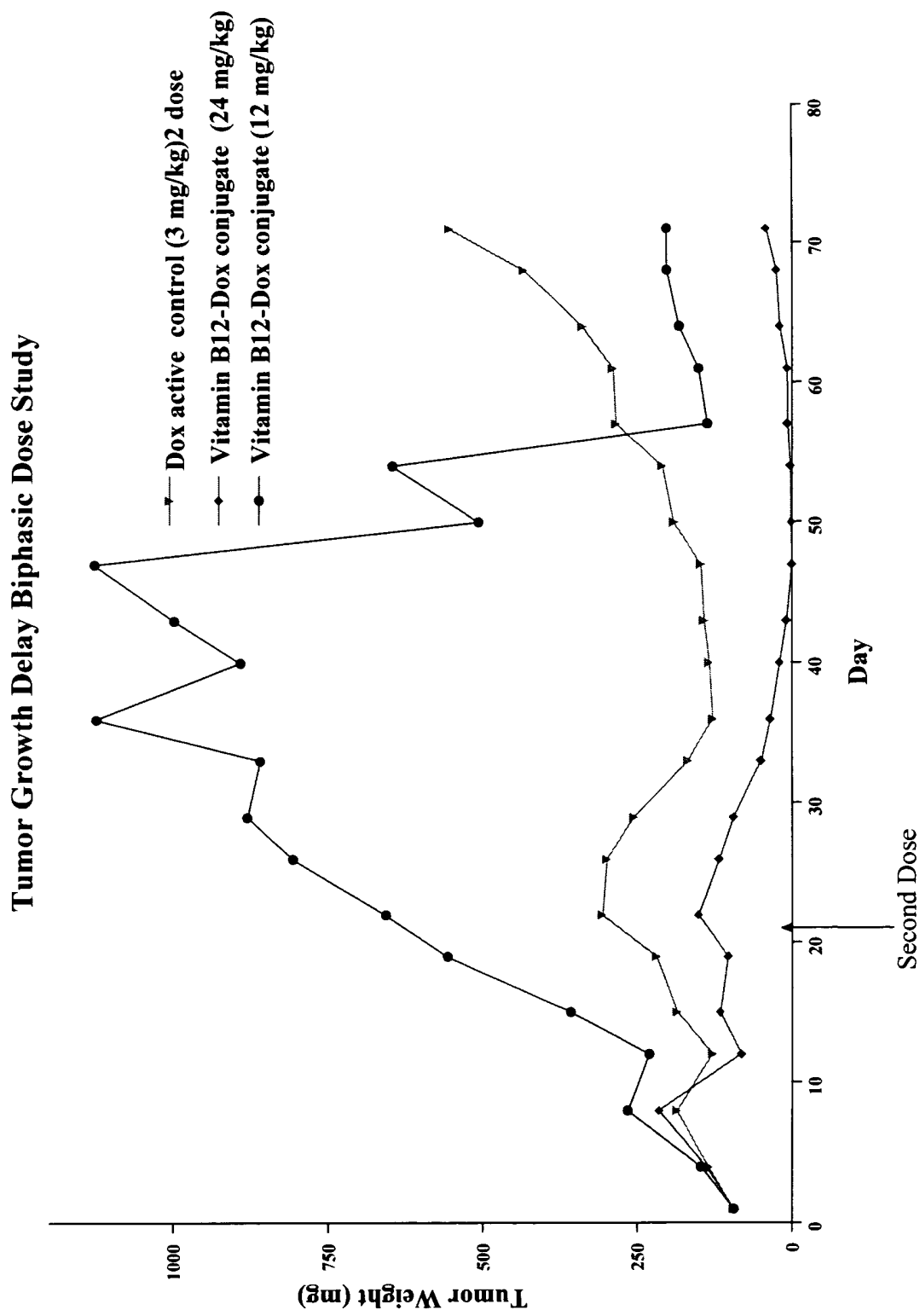# Viability of Normal Murine Lymph Node Cells

Av. % Viability

△ Doxorubicin + ConA
◇ CobalaRubicin-200 + ConA

[compound] (uM)
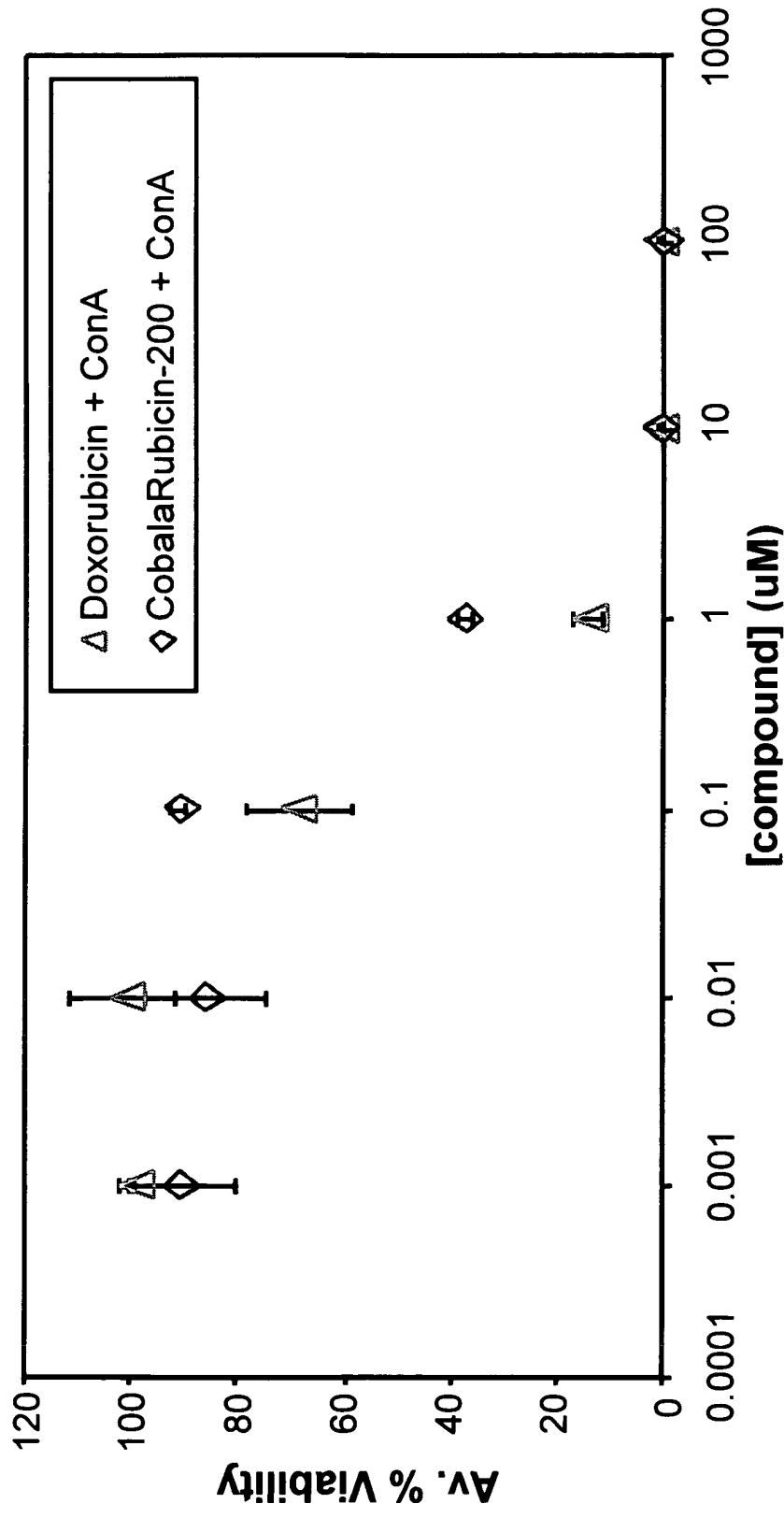
Effect of B12-DOX (stable linker) on viability of SK-N-MC cells treated for 48 hours
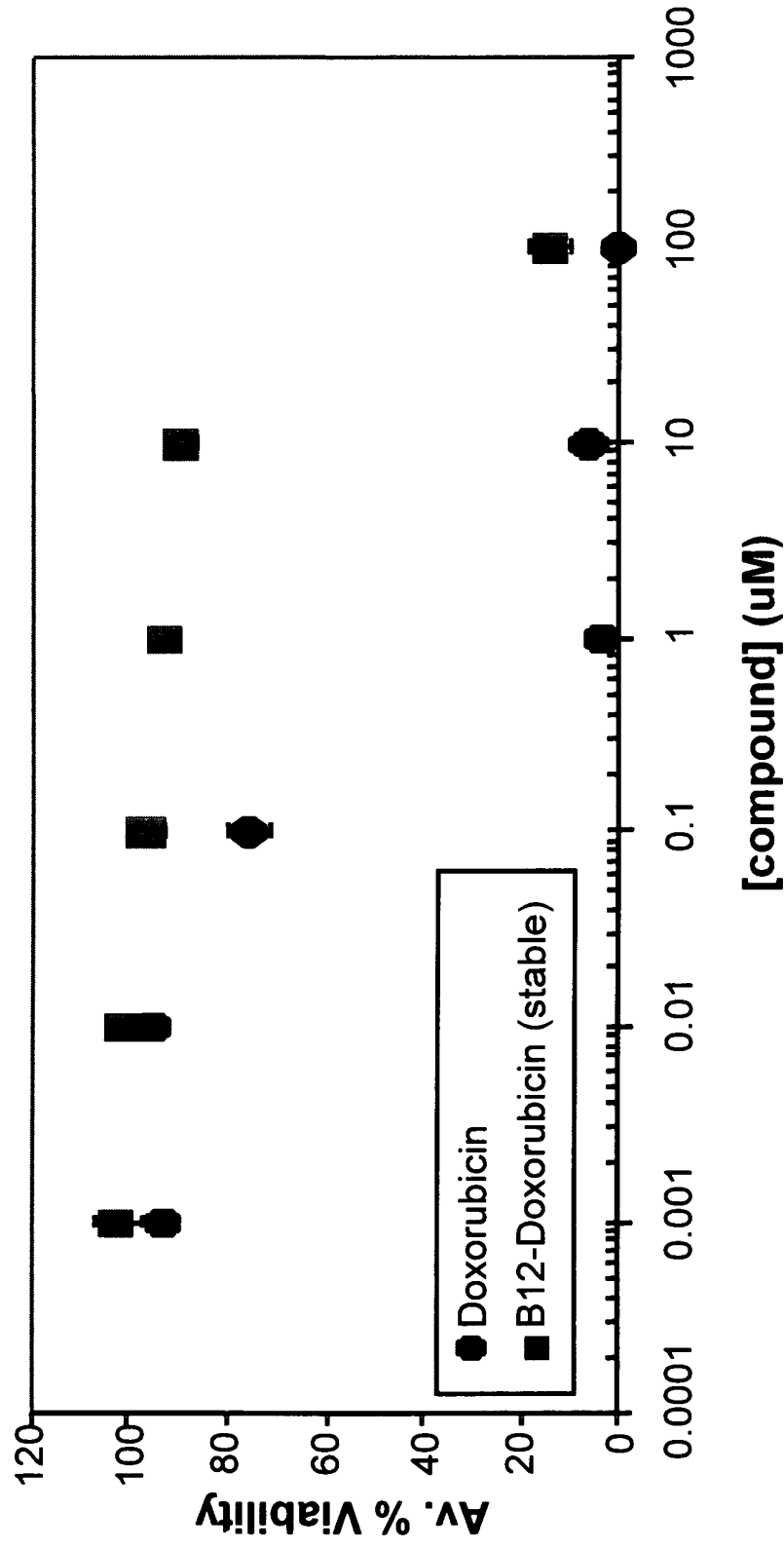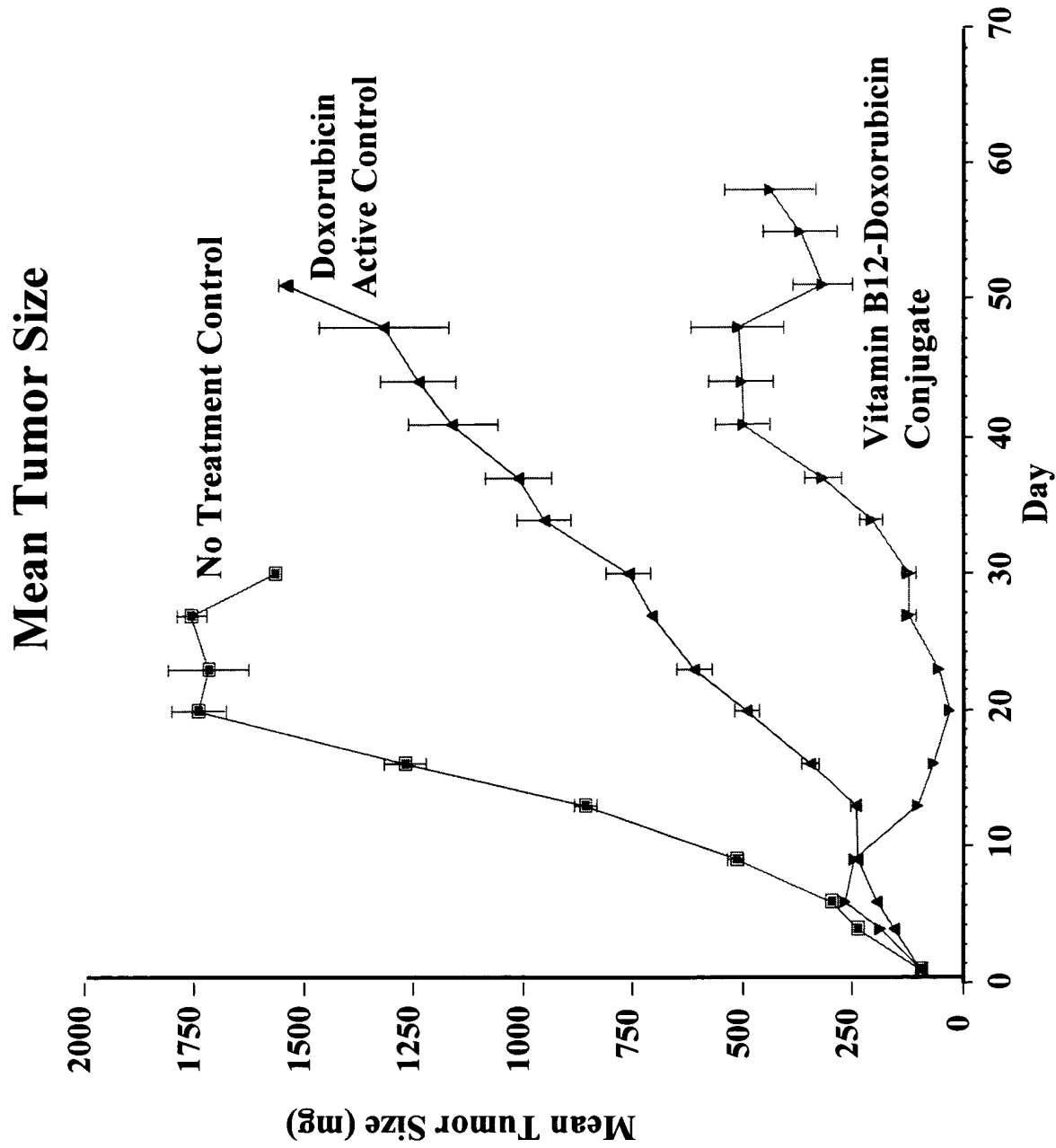
Mean Tumor Size

Percent Change in Body Weight

Tumor Growth Delay Biphasic Dose Study

Mean Body Weights

-0.8%

-8.4%

-10.5%

Body Weight (grms)

30
29
28
27
26
25
24
23
22
21
20

0   7   14   21

Days After First Day of Dosing

Saline
Doxorubicin Active Control
Vitamin B12-Doxorubicin conjugate
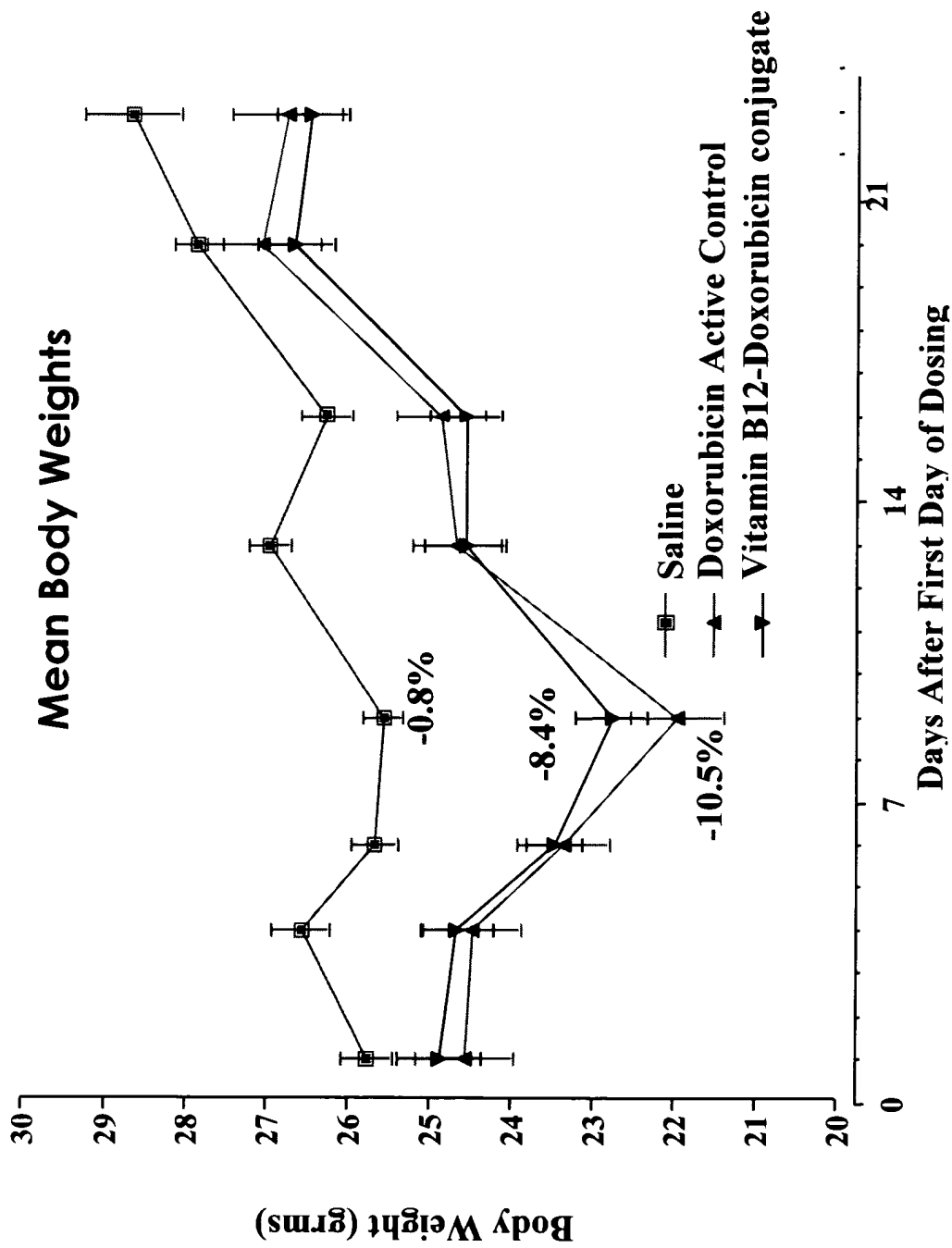
Summary of Body Weight Changes